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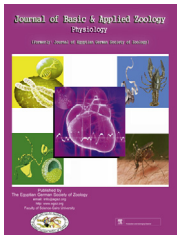

Ameliorative effect of antioxidants (vitamins C and E) against abamectin toxicity in liver, kidney and testis of male albino rats

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Abamectin;
Vitamin C;
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Abstract This study evaluated the effect of vitamins C and E as antioxidants on the physiological and histopathological changes induced by abamectin pesticide in liver, kidney and testis of male albino rats. Thirty male albino rats were divided into five groups of 6 rats each. First group served as control, while the second group received 10 mg/kg b.wt of abamectin orally, the third group received abamectin daily and 160 mg/kg b.wt of vitamin C two times per week. The fourth group received abamectin daily plus 50 mg/kg b.wt of vitamin E two times per week, while the fifth group received abamectin daily plus vitamins C and E two times per week. The experiment was conducted for six weeks. Abamectin was found to induce, hepato renal and testicular toxicity in rats, since the biochemical parameter of liver function (i.e. alanine amino transferase (ALT), aspartame amino transferase (AST), acid phosphatase (AP), glucose, total protein, albumin) and kidney function (i.e. creatinine, urea, uric acid, cholesterol and triglycerides) were highly affected. These effects were demonstrated by histopathological examination of liver, kidney and testis tissues. These observations were much reduced in the vitamin-treated groups.

In conclusion, it appears that vitamins C and E, or in combination (as antioxidants) ameliorate the hepato-renal and testicular toxicity of abamectin, but are not completely protective, especially in liver tissue.

Introduction

Abamectin (ABM) is a macrocyclic lactone product derived from the soil microorganism Streptomyces avermitilis. It is a mixture of avermectin containing about 80% avermectin B1a and 20% avermectin B1b (Burg et al., 1979; Fisher and Mrozik, 1989). These two components, B1a and B1b have similar biological and toxicological properties (Lankas and Gordon,1989). ABM is used as an insecticide and acaricide in many parts of the world.

Abamectin is nearly insoluble in water and has a strong to bind to soil particles. In the environment, ABM is quickly degraded (half life time 4–12 h.) by oxidative and

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photo-oxidative mechanisms when exposed to light in water or as thin film on biological surfaces (e.g. leaves) or when it binds to the soil particles and then exposed on glass plates (Wislockii et al., 1989). ABM is highly toxic to insects and fishes and may be highly toxic to mammals (Moline et al., 2000; Jençiç et al., 2006). Additionally, as a safe chemical in mammals, abamectin has been used as an anthelmintic agent in both animals and humans (Kaplan et al., 1994). Intoxication of abamectin may affect the function of hepatocytes although the permanent liver damage is usually not revealed immediately. In rats, abamectin led to an elevation in serum AST and nitric oxide (Hsu et al., 2001). It is known that the detoxification of the toxic materials which enter the body occurs mainly in the liver (Baisterri and Shaw, 1987). Therefore, liver can be used as an index for the toxicity of abamectin in vertebrate animals.

Previous study has shown that abamectin caused a significant increase in serum aspartate aminotransferase (AST), alanina aminotransferase (ALT), alkaline phosphatase (ALP) and acid phosphatase (AP) in rats treated with sub-acute dose (9.83 mg/kg b.wt for 21 days) and sub-chronic dose (5.93 mg/kg b.wt for 57 days). (Soliman et al., 2009). A dose of 1/10 or 1/100 of LD50 of abamectin led to an increase in the activity of AST and AP, whereas it caused a decrease in ALT activity, total protein, albumin and glucose concentration in serum of treated male rats in a dose-dependent manner, but ALP activity and cholesterol concentration remained unaltered (Eissa and Zidan, 2010).

Abamectin at a dose of 2–13 mg/animal/day for 14, 28 and 42 days was found to cause a significant increase in the glucose count and levels of AST and ALT in the liver of male and female albino rats (Khaldoun-Oularbi et al., 2013).

Histological examination of liver treated with 1/10 or 1/100 of LD50 of abamectin showed excess portal tract infiltration and a focus of dysplasia with cytological atypia in male albino rats (Eissa and Zidan, 2010). It has been postulated that treatment of male and female rats with abamectin caused vascular degeneration, hemorrhage, cellular infiltration, sinusoidal dilatation and foamy cytoplasm in the hepatocytes in the male albino rats (Khaldoun-Oularbi et al., 2013).

Abamectin may affect the kidney function parameters. It has been reported that administration of 1/4 LD50 of abamectin orally in male albino rats led to a significant increase in plasma levels of urea, uric acid, and creatinine, while that of glutathione-s-transferase(GST) and catalase enzymes were significantly decreased (El-shafey et al., 2011). Also it has been postulated that the administration of dietary dose of abamectin equivalent to 1/10 or 1/100 of the LD50 values for 30 days caused a significant increase in uric acid and creatinine concentrations of serum in the male albino rats (Eissa and Zidan, 2010). Administration of 30 mg/kg b.wt for 30 days three times per week or 10 mg/kg b.wt for 210 days once a week orally of abamectin in male albino rats resulted in a significant increase in the levels of plasma urea and creatinine but, a significant decrease in the levels of plasma albumin, total proteins and RNA was observed (Abd-Elhady and Abou-Elghar, 2013).

Recent studies revealed that administration of 30 mg/kg b.wt of abamectin orally for 30 days caused a decrease in the level of protein content, the activities of antioxidant enzymes and alkaline phosphatase (ALP) in male Wistar rats (Nasr et al. (2016).

Histopathological examination of the kidney tissue from male albino rats exposed to 1/10 or 1/100 of LD50 of abamectin for 30 days showed interstitial nephritis (Eissa and Zidan, 2010). The kidney tissue from male albino rats which was exposed to 1/10 or 1/30 of LD50 of abamectin orally showed marked necrosis of tubular cells, atrophy of the glomeruli and areas of interstitial infiltration of round cells (Abd-Elhady and Abou-Elghar, 2013).

Abamectin caused a significant increase in the level of plasma testosterone, but sperm count and sperm motility were significantly decreased in males albino rats (Eissa et al., 2003). It has been reported that subacute and sub chronic exposure of abamectin for 30 and 210 days respectively, resulted in a significant reduction in male albino rats that the number of springs was significantly reduced (Abd-Elhady and Abou-elghar, 2013).

Histopathological evaluation of the testes of albino rats which were ingesting abamectin at a dose of 1.19, 1.87 and 2.13 mg/animal/day for six weeks revealed several abnormalities including infiltration with congested blood vessels with marked hemorrhage and a significant accumulation of connective tissue surrounding the seminiferous tubules (Elbetieha and Da'as, 2003). The administration of low dose (1 mg/kg/day) for 1 week and high dose (4 mg/kg/day) for 6 weeks orally in male albino rats, resulted in disruption of spermatogenesis in a way that a tubule arrested in round spermatid stage consisting of immature germ cells with halo appearance in their nuclei, tubule with disrupted spermatogenesis comprising abnormal gametes and consisting of multinuclear giant cell (Celik-Ozenci et al., 2011). Oral administration of abamectin at a dose of 30 mg/kg b.wt three times a week for 30 days and 10 mg/kg b.wt for 210 days, once a week showed degeneration of spermatogonia cells lining seminiferous tubules and lumen contains fewer spermatozoa, necrosis of spermatogonia cells lining seminiferous tubules associated with peritubular edema and the lumen contains a decreased number of spermatogenesis elements (Abd-Elhady and Abou-elghar, 2013).

The body has several mechanisms to counteract the damage caused by free radicals. The basic and the most important defense mechanism of the body are antioxidant agents (Abdollahi et al., 2004). The term antioxidant is any substance that delays, prevents or removes oxidative damage to a target molecule (Halliwell, 2007). Pesticides have been extensively studied for their toxic potentials. Pesticide induced oxidative stress has been the focus of toxicological research for over decade as a possible mechanism of toxicity. Studies have established oxidative stress in humans and animals result from various agents in the group and are associated with their toxic manifestation (Uchendu et al., 2012).

It has been established that organophosphate pesticides which have been widely used in agriculture to enhance food production a public health to control nuisance bests may cause oxidative stress through excessive production of reactive oxygen species resulting in an imbalance between the production of free radicals and cellular antioxidants (Milatovic et al., 2006). So, it should be noted that the information about the toxic effects of abamectin induced oxidative stress in the literatures are very rare. It has been stated that antioxidant may ameliorate, protect and remove the oxidative damage to a target organ or molecule (El-Shenawy and Al-Ghamdi, 2014).

The major natural antioxidant which are derived from the natural sources by dietary intake are vitamins A, C, E and carotenoids (Heistad,2006). Accordingly, interest has recently grown in the role of the natural antioxidant as a strategy to

prevent oxidative damage as a factor in the pathophysiology and histopathology of various health disorders (Shireen et al., 2008; Budin et al., 2011). Among antioxidants, ascorbic acid (vitamin C) and tocopherol (vitamin E) used as a nutritional supplements, are the essential elements in almost all biological systems.

Vitamin C (ascorbic acid), it is a water-soluble chain-breaking antioxidant (Sauberlich, 1994). It is one of the most widely available and affordable non-enzymatic antioxidant molecules that have been used to mitigate oxidative damage (Naidu, 2003). It readily scavenges physiological ROS as well as reactive nitrogen species "RNS" (Carr and Frei, 1999).

Fruits, vegetable and organ meats (e.g. liver and kidney) are generally the best sources of ascorbic acid, muscle meats and most seeds do not contain significant amount of ascorbic acid (Combs, 1992).

It has been reported that vitamin C ameliorates organophosphate pesticide-induced hematological and biochemical alterations in humans and animals (Ambali et al., 2007, 2011b; Aly et al., 2010; Karmmon et al., 2011). This readily available, cheap and relatively non-toxic antioxidant possesses great benefit in the amelioration of toxic effects by most xenobiotics (Uchendu et al., 2012).

Vitamin E (α-tocopherol) it is a lipid soluble antioxidant present in all cellular membranes protecting against lipid peroxidation (Machlin, 1980). It functions as a chain-breaking antioxidant by preventing chain initiation and propagation of free radical reaction and lipid peroxidation in cellular membrane (Kamal-Eldin and Appelqvist, 1996). In addition to its antioxidant function, vitamin E supplementation influences the cellular response to oxidative stress through modulation of signal-transduction pathway (Azzi et al., 1992). Also, vitamin E functions as membrane stabilizer (Truber and Packer, 1995; Clarke et al., 2008). Vegetable oil and wheat-germ oil are rich in vitamin E. Because of its hydrophobicity, dietary vitamin E requires special transport mechanisms in aqueous environment of the plasma, body fluids and cells.

It must be stated that the information about the influences of vitamin E on the abamectin-induced toxicity in animals and humans are very scarce. However, it has been reported that vitamin E supplementation protected against chlorpyrifos (CPF) induced hematobiochemicals toxicity in animal mode (Ambali et al., 2010e) and sensor motor and cognitive changes (Ambali and Ayo, 2011). Also, it has been documented that vitamin E had a beneficial effects against other organophosphates pesticide-induced toxicity.

It has been documented that the combination of vitamins C and E had a protective effect against CPF-induced hematological and biochemical toxicity in albino rats (Gultekin et al., 2001;Ahmed et al., 2010; Ambali et al., 2010d; Ambali et al., 2010e). Several studies have also demonstrated their beneficial effects against OPS-induced hepatotoxicity and ultrastructural changes in rats (Kalender et al., 2005; El-Shenawy et al., 2009; El-Shenawy, 2010a,b). Also, the combination of vitamins C and E had a protective effect against malathion-induced testicular toxicity in male Wistar rats (Uzun et al., 2009). Moreover, vitamins C and E can act in a preventive way and moderate the effect of OP (endosulfan) on lipid peroxidation in the adult rat brain (Zervos et al., 2011). So, it must be noted, again, that the impact of the combination of vitamins C and E on abamectin-induced toxicity in literature are very rare.

The objective of the present work is to investigate the biochemical and histological changes induced by abamectin in the liver, kidney and testis of male albino rats who had been given abamectin orally. Also, the present study is intended to evaluate whether the toxic effects of abamectin on the biochemical and histological parameter examined can be ameliorated by treating with vitamin C, vitamin E and co-treatment with vitamins C and E.

Materials and methods

Animals

Thirty sexually mature male albino rats (*Rattus rattus*) weighing approximately (160–180 g) were obtained from the animal house of zoology department, Faculty of Science, Sohag University, Egypt. The animal were housed in stainless steel cages, fed a standard laboratory diet and water *ab libitum*, exposed to 12 h. light/dark cycle, and maintained at a laboratory temperature of 25 ± 5°C. The animals were quarantined for 2 weeks before beginning the experiments. All rats were handled in accordance with the standard guide for the care and use of the laboratory animals.

Chemicals

Abamectin was obtained from Syngenata Agro Co., Switzerland, vitamin C (ascorbic acid) and vitamin E (α-tocopherol) were supplied by Loba Chemicals, India. All chemicals used were of analytical grade.

Animal treatments schedule

The rats were divided into five groups, namely, the control group (G1), abamectin treated group (G2), abamectin and vitamin C treated group (G3), abamectin and vitamin E treated group (G4), abamectin and vitamin C plus vitamin E treated group (G5). The number of rats in each group was 6. Abamectin was dissolved in dist. Water and administered orally (10 mg/kg body wt.) daily. Vitamin C was dissolved in dist. water and administered orally (160 mg/kg body wt.) two times per week, whereas vitamin E was dissolved in sesame oil and administered by subcutaneous injections (50 mg/kg body wt.) two times per week. The study was conducted for six weeks.

Blood sampling

At the end of experimental period (six weeks), blood samples were individually collected from each rat, immediately after decapitation, from the heart into dry clean tubes containing EDTA as anticoagulant, then were centrifuged at 3000 rpm. for 20 min to obtain plasma. The obtained plasma was stored at −20 °C until use for biochemical analysis.

Biochemical analysis

The activities of some biochemicals parameters representing liver and kidney functions were determined in rats' blood plasma colorimetrically as follows: Alanine and aspartate

aminotransferase (ALT and AST) activities were determined according to the method of Reitman and Frankel (1957), acid phosphatase (AP) according to the method of Moss (1984), total protein according to the method of Henry (1964), glucose and albumin were measured according to the methods of Hyvarinen and Nikkila (1962) and Doumas and Biggs (1976), respectively. Creatinine, urea, uric acid, cholesterol and triglycerides were estimated according to the methods of Henry et al. (1974), Chaney et al. (1962), Patton and Crouch (1972), Barham and Trinder (1972), Watson (1960); and Bablock et al. (1988), respectively. All determinations were done using USA spectrophotometer UVA 2300.

Tissue sampling

The influence of abamectin on the histopathology of liver, kidney and testis was investigated. At the end of the experiment (six weeks), liver, kidney, and testis from each scarified rat were removed, trimmed from excess fat and were fixed in neutral buffer formalin and prepared for histopathological examination according to Drury and Wallington (1980).

Statistical analysis

Results are presented as mean ± SE for comparison of different experimental animal groups and control ones. The results were statistically analyzed by a one way ANOVA. *P*-value >0.05 was considered significant.

Results

Effect of different treatments on liver function parameters

Liver is often the primary target for toxic effect of xenobiotics. It is known that the detoxification of the toxic materials which enter the body occurs mainly in the liver. Therefore, liver can be used as an index for the toxicity of xenobiotics. So, the activities of some enzymes representing liver function i.e. ALT, AST, AP, glucose, total proteins and albumin were determined in rats treated with abamectin and the control group. Also, the ameliorative effects of vitamins C and E against the abamectin toxicity were determined. Relative to the control, abamectin elevated the plasma level of ALT significantly ($p < 0.03$), whereas abamectin combined with vitamin C, and abamectin combined with vitamin C plus vitamin E resulted in a nonsignificant increase in the ALT activity. However, abamectin combined with vitamin E did not have any effect (Fig. 1).

Abamectin caused a highly significant ($p < 0.0002$) increase in the plasma level of AST. Also, abamectin combined with vitamin C led to a significant ($p < 0.05$) increase in AST activity, while abamectin combined with vitamin E led to a nonsignificant decrease in the level of AST, but abamectin combined with vitamin C plus vitamin E caused a nonsignificant increase in the activity of AST (Fig. 2). Similar to the effect on the ALT and AST activities, abamectin increased the plasma level of AP significantly ($p < 0.03$), whereas acid phosphatase activity (AP) remained unaltered under the effects of abamectin combined with either vitamin C or vitamin E or both (Fig. 3).

Abamectin resulted in a highly significant ($p < 0.000009$) increase in the plasma level of glucose, relative to the control. However, abamectin combined with vitamin C and abamectin combined with vitamin C plus vitamin E caused a highly significant ($p < 0.00003$, $p < 0.0000001$, respectively) decrease in the plasma level of glucose, whereas abamectin combined with vitamin E caused a nonsignificant decrease in the same parameter (Fig. 4). Abamectin resulted in a highly significant ($p < 0.0003$) increase in the plasma level of total protein, like that of glucose. Also, abamectin combined with either vitamin C or vitamin E resulted in a significant ($p > 0.01$; $p > 0.05$, respectively) increase in the plasma level of total proteins, in contrast to that of glucose. But, in contrast to that of glucose, abamectin combined with vitamin C plus vitamin E resulted in a nonsignificant increase in the level of plasma total proteins (Fig. 5). Like that of plasma total proteins, abamectin caused a significant ($p < 0.02$) increase in the plasma level of albumin. Also, abamectin combined with vitamin C resulted in a significant ($p < 0.005$) increase in the activity of albumin. But, abamectin combined with vitamin E and abamectin combined with both vitamin C and vitamin E resulted in a nonsignificant increase in the plasma level of albumin (Fig. 6).

Effect of different treatments on kidney function parameters

Results in Fig. 7 indicated that abamectin resulted in a significant ($p < 0.02$) decrease in the plasma level of creatinine, relative to the control, whereas, abamectin combined with either vitamin C or vitamin E, and with both vitamin C and vitamin E resulted in a nonsignificant decrease in the same parameter. Abamectin caused a significant ($p < 0.003$) increase in the plasma level of urea. Also, abamectin combined with either vitamin C or vitamin E caused a significant ($p < 0.01$) increase in the same parameter, whereas the same parameter under the effect of abamectin combined with vitamin C plus vitamin E was nearly the same as control (Fig. 8). Abamectin and abamectin combined with vitamin E caused a significant ($p < 0.01$) increase in the plasma level of uric acid like that of urea. But, abamectin combined with vitamin C caused a nonsignificant increase in the same parameter. Unlike that of urea, abamectin combined with vitamins C and E resulted in a highly significant ($p < 0.00000002$) increase in the plasma level of uric acid (Fig. 9).

As indicated in Fig. 10, abamectin resulted in a significant ($p < 0.05$) decrease in the plasma level of cholesterol. However, abamectin combined with vitamin C or E, and with both vitamins C and E resulted in a nonsignificant increase in the same parameter. In contrast to that of cholesterol, abamectin, abamectin combined with vitamin C or E caused a highly significant ($p < 0.0002$) increase in the plasma level of triglycerides. But, abamectin combined with vitamin C plus E caused a significant ($p < 0.002$) decrease in the same parameter (Fig. 11).

Histopathological examination

Histopathological changes were observed in all examined organs of abamectin treated group, abamectin and vitamin C treated group, abamectin and vitamin E treated group, and abamectin combined with vitamin C and vitamin E treated group. Control group show normal hepatocytes cells which

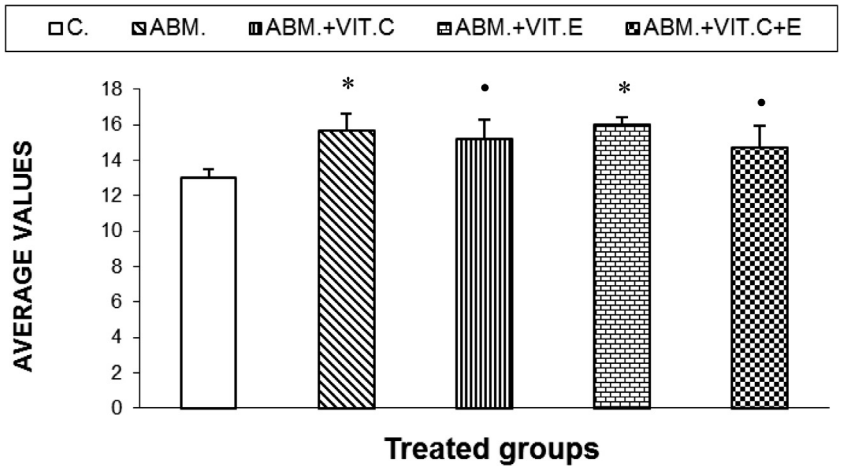

Figure 1 Effects of abamectin, abamectin plus Vit.C, abamectin plus Vit.E, and abamectin combined with Vit.C plus Vit.E on the level of plasma ALT (U/L) after six weeks treatment of rats (*Rattus rattus*). The number of rats in each series was 6. $^{\bullet}p < 0.05$ (non significant). $^{*}P > 0.05$ (significant difference with respect to control group). C., Control; ABM., Abamectin; ABM. + Vit.C, Abamectin + Vitamin C; ABM. + Vit.E, Abamectin + Vitamin E; ABM. + Vit.C + Vit.E, Abamectin + Vitamin C + Vitamin E.

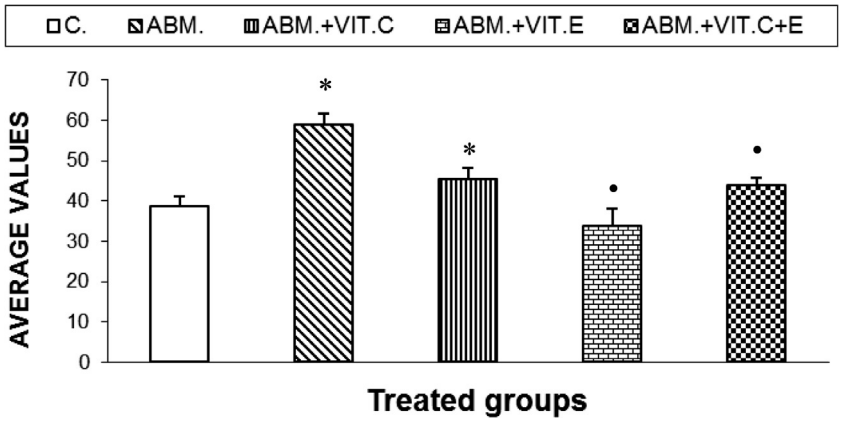

Figure 2 Effects of abamectin, abamectin plus Vit.C, abamectin plus Vit.E, and abamectin combined with Vit.C plus Vit.E on the level of plasma AST (U/L) after six weeks treatment of rats (*Rattus rattus*). The number of rats in each series was 6. $^{\bullet}p < 0.05$ (Non significant). $^{*}P > 0.05$ (Significant difference with respect to control group). C., Control; ABM., Abamectin; ABM. + Vit.C, Abamectin + Vitamin C; ABM. + Vit.E, Abamectin + Vitamin E; ABM. + Vit.C + Vit.E, Abamectin + Vitamin C + Vitamin E.

were well arranged separated from sinusoids, and uniformly stained (Plate 1A). In contrast to normal histological examination of the liver tissues of the untreated control, marked edema (E) and dilation of Disse's space were noticed in abamectin treated rats (Plate 1B). In abamectin and vitamin C treated animals, most of the hepatocytes appeared to somewhat normal but associated with dilation of the blood vessels (D) (Plate 1C). Also, the hepatocytes appeared with normal architecture, but with the presence of inflammatory cell infiltrate in abamectin and vitamin E treated group (Plate 1D). Necrobiosis changes of few hepatocytes were observed in abamectin combined with vitamin C plus vitamin E treated animals (Plate 1E).

The control rat kidney section show normal renal histological architecture of the kidney glomerular and surrounding tubules (2A). Abamectin treated group showed glomerulus necrosis (GN) and tubular necrobiosis (TN) associated with hemorrhage in the renal cortex (HR) (Plate 2B). The glomeruli (G) and the renal tubules (RT) appeared more or less normal in the abamectin and vitamin C treated animals (Plate 1C). Also, in the abamectin and vitamin E treated group, the glomeruli and the renal tubules (RT) appeared more or less normal with the presence of few inflammatory cells (F) around glomeruli (Plate 1D). The section of kidney from the rats treated with abamectin and vitamin C plus vitamin E showed that the histological picture of the kidney appeared more or less normal (Plate 1E).

The histological examination of testis showed that the control group appeared with normal testicular histology with all successive stages of spermatogenesis (Plate 3A). There were intratubular edema (TE) and degeneration in some spermatogenic cells (DS) with the presence of few number of spermatozoa in the testis of the abamectin treated animals (Plate 3B). The testis section from the rats treated with abamectin plus vitamin C (Plate 3C). The rats treated with abamectin plus vitamin E (Plate 3D) and the rats treated with abamectin and vitamin C plus Vitamin E (Plate 3E) showed normal histological structure of testis.

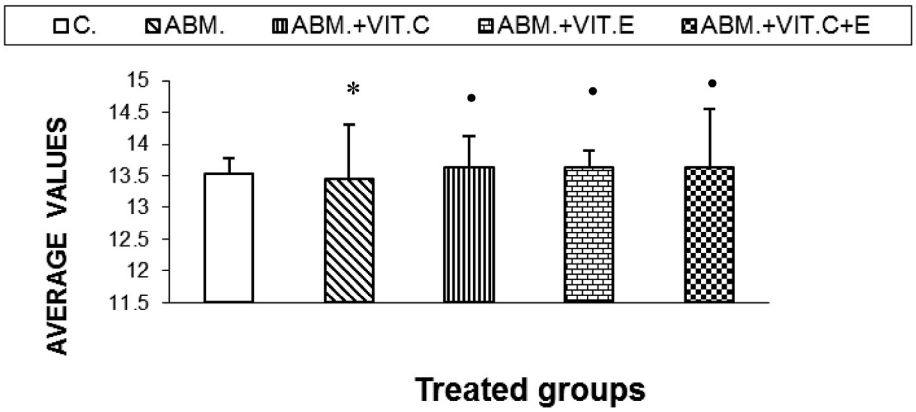

Figure 3 Effects of abamectin, abamectin plus Vit.C, abamectin plus Vit.E, and abamectin combined with Vit.C plus Vit.E on the level of plasma AP (U/L) after six weeks treatment of rats (*Rattus rattus*). The number of rats in each series was 6. $^{\bullet}p < 0.05$ (non significant). $^{*}P > 0.05$ (significant difference with respect to control group). C., Control; ABM., Abamectin; ABM. + Vit.C, Abamectin + Vitamin C; ABM. + Vit.E, Abamectin + Vitamin E; ABM. + Vit.C + Vit.E, Abamectin + Vitamin C + Vitamin E.

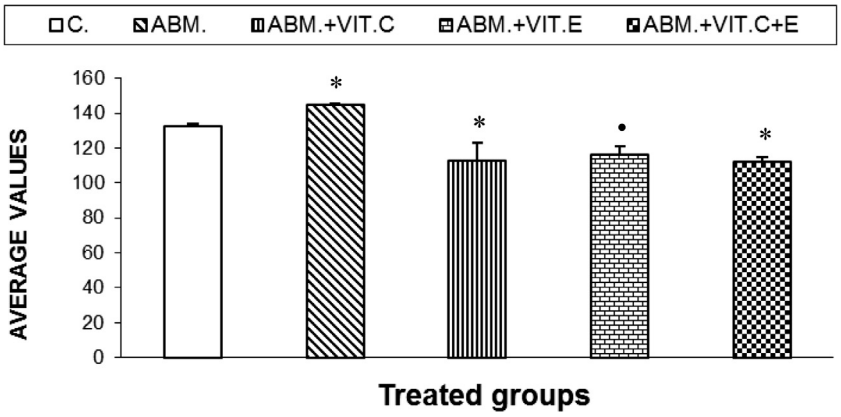

Figure 4 Effects of abamectin, abamectin plus Vit.C, abamectin plus Vit.E, and abamectin combined with Vit.C plus Vit.E on the level of plasma GLUCOSE (mg/dL) after six weeks treatment of rats (*Rattus rattus*). The number of rats in each series was 6. $^{\bullet}p < 0.05$ (non significant). $^{*}P > 0.05$ (significant difference with respect to control group). C., Control; ABM., Abamectin; ABM. + Vit.C, Abamectin + Vitamin C; ABM. + Vit.E, Abamectin + Vitamin E; ABM. + Vit.C + Vit.E, Abamectin + Vitamin C + Vitamin E.

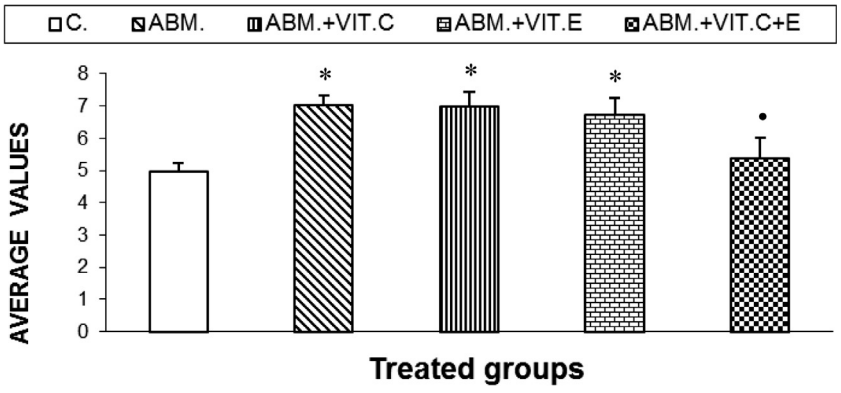

Figure 5 Effects of abamectin, abamectin plus Vit.C, abamectin plus Vit.E, and abamectin combined with Vit.C plus Vit.E on the level of plasma PROTEIN (g/dL) after six weeks treatment of rats (*Rattus rattus*). The number of rats in each series was 6. $^{\bullet}p < 0.05$ (Non significant). $^{*}P > 0.05$ (Significant difference with respect to control group). C., Control; ABM., Abamectin; ABM. + Vit.C, Abamectin + Vitamin C; ABM. + Vit.E, Abamectin + Vitamin E; ABM. + Vit.C + Vit.E, Abamectin + Vitamin C + Vitamin E.

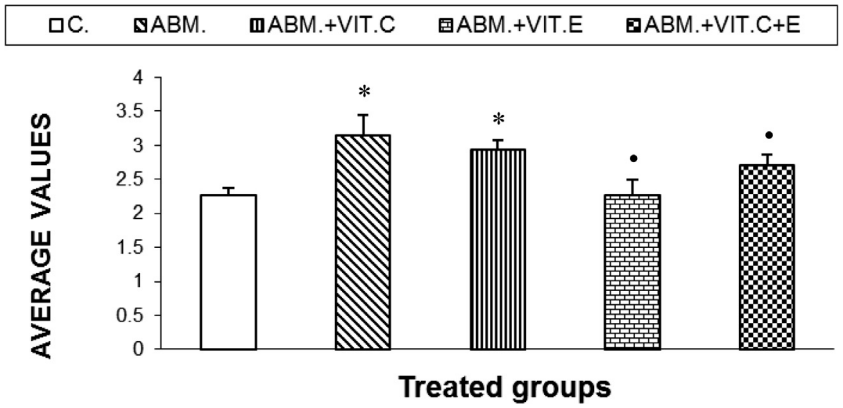

Figure 6 Effects of abamectin, abamectin plus Vit.C, abamectin plus Vit.E, and abamectin combined with Vit.C plus Vit.E on the level of plasma ALBUMIN (g/dL) after six weeks treatment of rats (*Rattus rattus*). The number of rats in each series was 6. $^{\bullet}p < 0.05$ (non significant). $^{*}P > 0.05$ (significant difference with respect to control group). C., Control; ABM., Abamectin; ABM. + Vit.C, Abamectin + Vitamin C; ABM. + Vit.E, Abamectin + Vitamin E; ABM. + Vit.C + Vit.E, Abamectin + Vitamin C + Vitamin E.

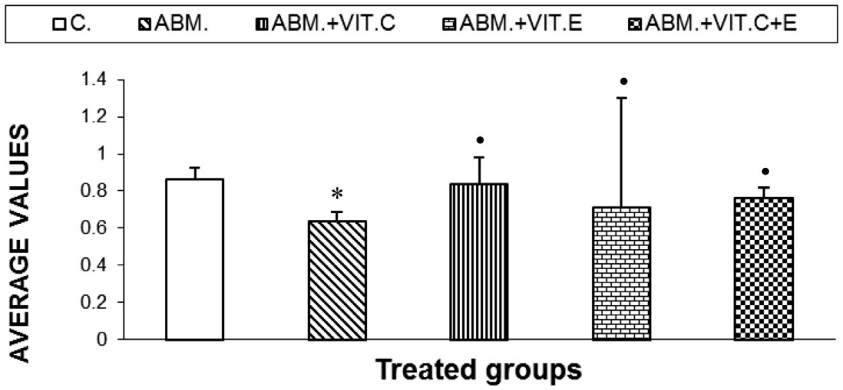

Figure 7 Effects of abamectin, abamectin plus Vit.C, abamectin plus Vit.E, and abamectin combined with Vit.C plus Vit.E on the level of plasma CREATININE (mg/dL) after six weeks treatment of rats (*Rattus rattus*). The number of rats in each series was 6. $^{\bullet}p < 0.05$ (non significant). $^{*}P > 0.05$ (significant difference with respect to control group). C., Control; ABM., Abamectin; ABM. + Vit.C, Abamectin + Vitamin C; ABM. + Vit.E, Abamectin + Vitamin E; ABM. + Vit.C + Vit.E, Abamectin + Vitamin C + Vitamin E.

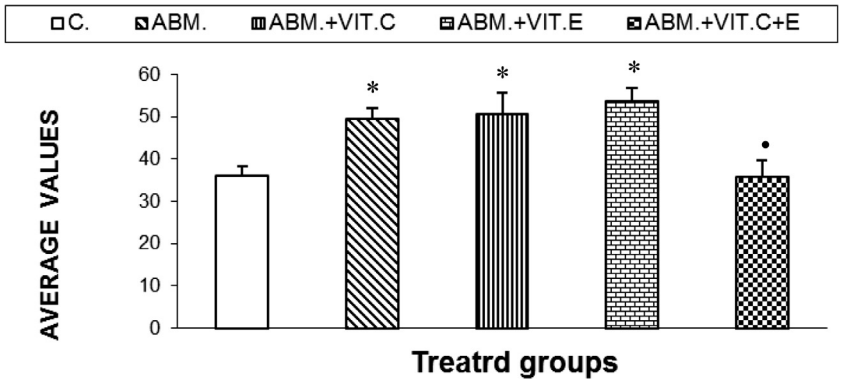

Figure 8 Effects of abamectin, abamectin plus Vit.C, abamectin plus Vit.E, and abamectin combined with Vit.C plus Vit.E on the level of plasma UREA (mg/dL) after six weeks treatment of rats (*Rattus rattus*). The number of rats in each series was 6. $^{\bullet}p < 0.05$ (non significant). $^{*}P > 0.05$ (significant difference with respect to control group). C., Control; ABM., Abamectin; ABM. + Vit.C, Abamectin + Vitamin C; ABM. + Vit.E, Abamectin + Vitamin E; ABM. + Vit.C + Vit.E, Abamectin + Vitamin C + Vitamin E.

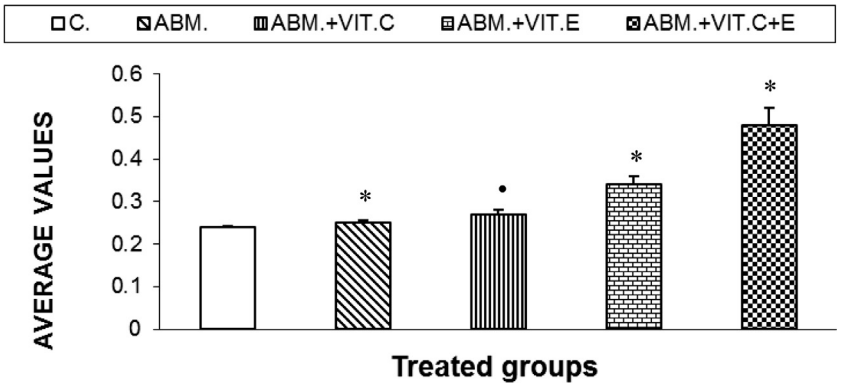

Figure 9 Effects of abamectin, abamectin plus Vit.C, abamectin plus Vit.E, and abamectin combined with Vit.C plus Vit.E on the level of plasma URIC ACID (mg/dL) after six weeks treatment of rats (*Rattus rattus*). The number of rats in each series was 6. $^{\bullet}p < 0.05$ (non significant). $^{*}P > 0.05$ (significant difference with respect to control group). C., Control; ABM., Abamectin; ABM. + Vit.C, Abamectin + Vitamin C; ABM. + Vit.E, Abamectin + Vitamin E; ABM. + Vit.C + Vit.E, Abamectin + Vitamin C + Vitamin E.

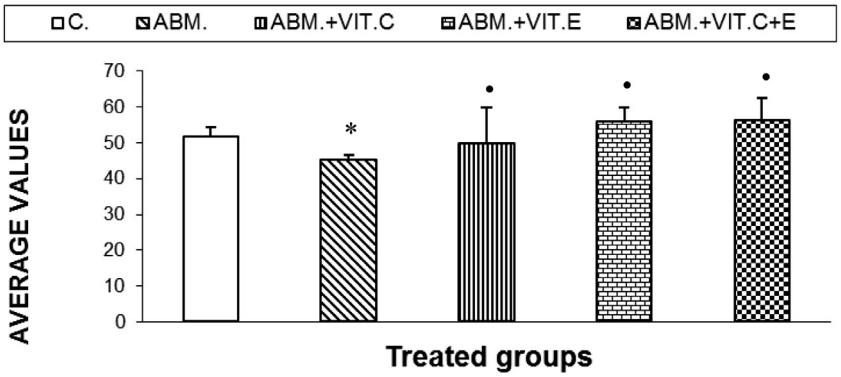

Figure 10 Effects of abamectin, abamectin plus Vit.C, abamectin plus Vit.E, and abamectin combined with Vit.C plus Vit.E on the level of plasma CHOLESTEROL (mg/dL) after six weeks treatment of rats (*Rattus rattus*). The number of rats in each series was 6. $^{\bullet}p < 0.05$ (non significant). $^{*}P > 0.05$ (significant difference with respect to control group). C., Control; ABM., Abamectin; ABM. + Vit.C, Abamectin + Vitamin C; ABM. + Vit.E, Abamectin + Vitamin E; ABM. + Vit.C + Vit.E, Abamectin + Vitamin C + Vitamin E.

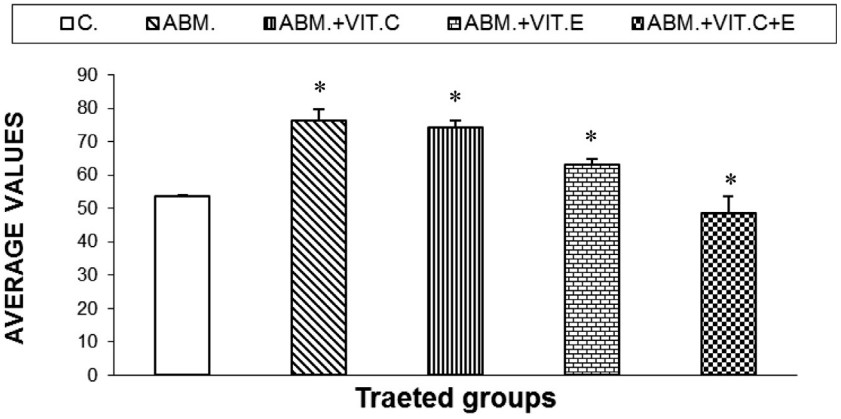

Figure 11 Effects of abamectin, abamectin plus Vit.C, abamectin plus Vit.E, and abamectin combined with Vit.C plus Vit.E on the level of plasma TRIGLYCERIDES (mg/dL) after six weeks treatment of rats (*Rattus rattus*). The number of rats in each series was 6. $^{\bullet}p < 0.05$ (non significant). $^{*}P > 0.05$ (significant difference with respect to control group). C., Control; ABM., Abamectin; ABM. + Vit.C, Abamectin + Vitamin C; ABM. + Vit.E, Abamectin + Vitamin E; ABM. + Vit.C + Vit.E, Abamectin + Vitamin C + Vitamin E.

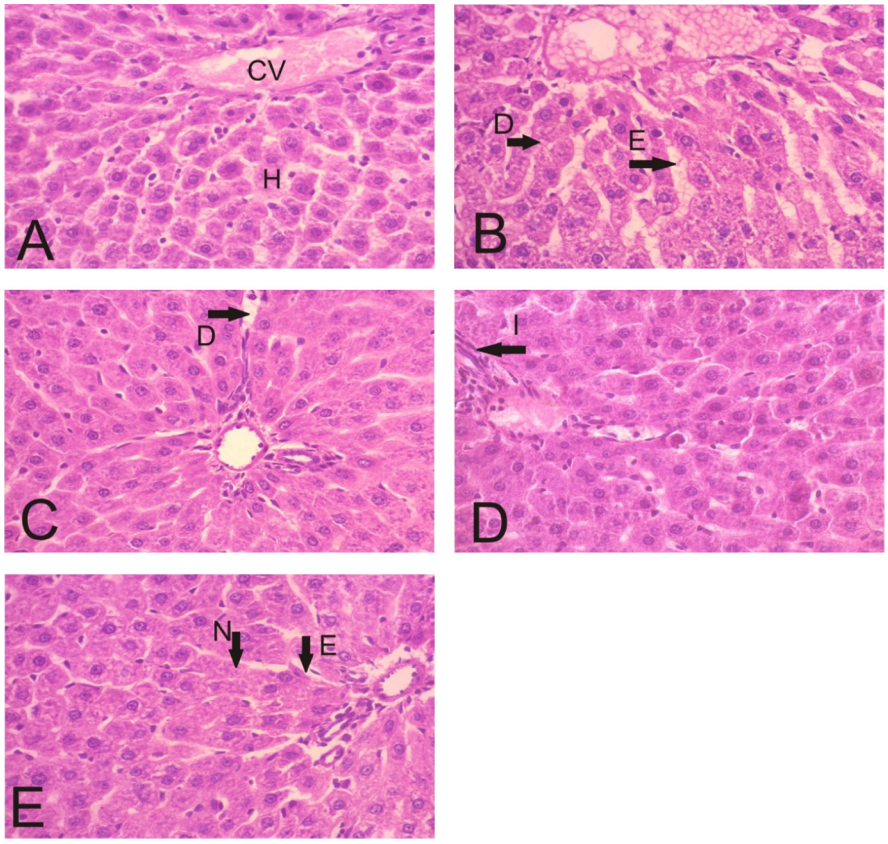

Plate 1 Photomicrograph of liver section from rats of the control group (G1) showing normal morphological architecture of the central vein (CV) and surrounding hepatocytes (H), (A); of the second group (G2) which was treated with abamectin showing edema (E) and dilation of Disse's space (D), (B); of the third group (G3) which was treated with abamectin combined with vitamin C showing most of the hepatocytes appeared to somewhat normal but associated with dilation of the blood vessels (D), (C); of the fourth group (G4) which was treated with abamectin combined with vitamin E showing that hepatocytes appeared with normal architecture associated with the presence of inflammatory cell infiltrate (I), (D); of the fifth group (G5) which was treated with abamectin combined with vitamin C plus vitamin E showing necrobiosis changes of few cells of hepatocytes (N), (E). (H&E) (40×).

Discussion

The present study provides additional information on abamectin-induced toxicity in the rats, after oral administration. The oral administration seems to be the most relevant in long-term real administration for the general population, due to residues in the food (Cometa et al., 2007). Abamectin pesticide was chosen in this study because it has been reported that it is used extensively all over the world including Egypt (Khaldoun-oularbi et al., 2013; Sadek and Shabeen, 2015).

The present study was designed to explore the toxic effects of abamectin on the histological structure of liver, kidney and testis. Also the protective effect of vitamin C, E, and vitamin C combined with vitamin E against abamectin toxicity was examined. Moreover, the influence of abamectin on liver and kidney function was investigated, also.

The weight

During the study period, there were no clinical signs of toxicity in any treatment group. At the end of the experimental study (six weeks) and after treatments, there was a nonsignificant increase in the body weight of abamectin treated group, relative to the control group. This result is in accordance with that reported by Khaldoun-Oularbi et al. (2013), while, there were a significant increase in the body weight of both vitamin C and E treated groups.

Liver

Liver can be used as an index for abamectin toxicity. It has been reported that abamectin may have a harmful effect on the hepatic cells (Eman and AbdAlla, 2000; Soliman et al., 2009). Also, it was found that liver contained high residues of abamectin (Roudaut, 1998). Thus, this may led to the damage of hepatocytes which is associated with the alterations of their organelles and morphological change resulting in changes of various biochemical functions of the liver. So, the activities of some enzymes and levels of certain biochemical parameters representing liver function i.e. ALT, AST, AP, glucose, total protein and albumin were affected (Eissa and Zidan, 2010).

In the present study, it was found that the concentration of plasma ALT increased significantly in rats treated with abamectin, a result in agreement with that of Hsu et al. (2001) who showed elevated levels of cytosolic enzyme of the hepatocytes. Moreover, it has been stated that abamectin resulted in a

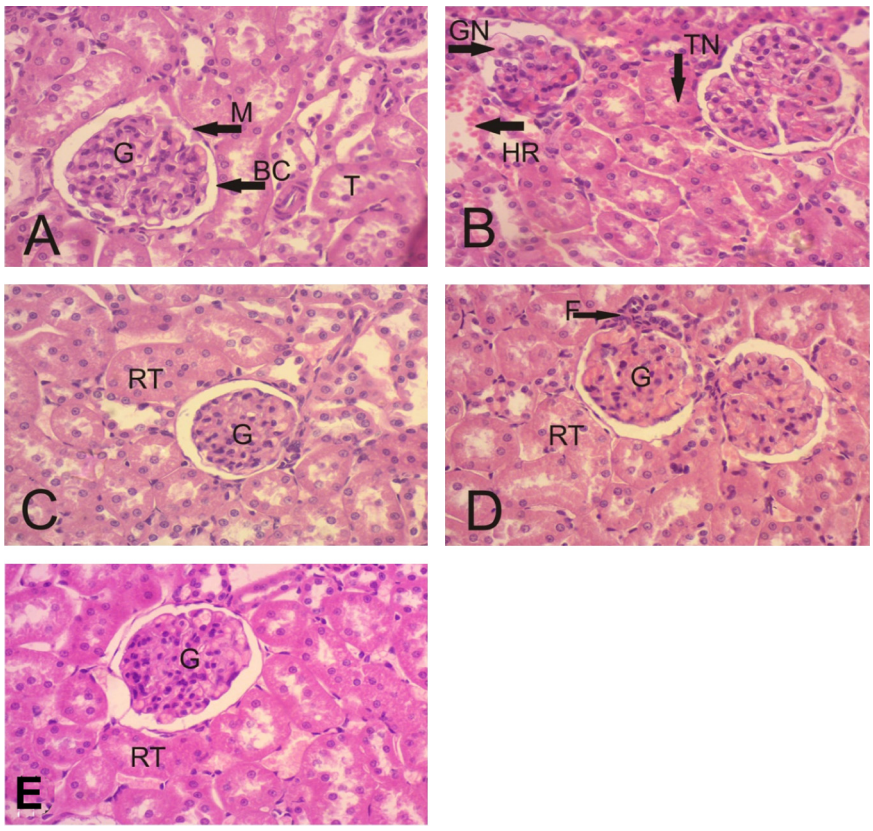

Plate 2 Photomicrograph of kidney section from rats of the control group (G1) showing normal renal architecture of the glomerular, surrounding tubules (T). The cortex contains Malpighian corpuscles (M), Bawman's capsule (BC) surrounding a capillary network of glomerulus (G), (A); of the second group (G2) which was treated with abamectin showing glomerulus necrosis (GN) associated with hemorrhage in the renal cortex (HR) and tubular necrobiosis (TN), (B); of the third group (G3) which was treated with abamectin combined with vitamin C showing the glomeruli (G) and the renal tubules (RT) appeared more or less normal, (C); of the fourth group (G4) which was treated with abamectin combined with vitamin E showing the glomeruli (G) and the renal tubules (RT) appeared more or less normal with the presence of few inflammatory cells (F) around glomeruli, (D); of the fifth group (G5) which was treated with abamectin combined with vitamin C plus vitamin E showing histological picture of the kidney appeared more or less normal, (E). (H&E) (40×).

significant increase in the activity of ALT in male albino rats (Soliman et al., 2009; Abd-Elhady and Abou-Elghar, 2013). Also, Abamectin resulted in a highly significant increase in the levels of AST, compared with that of control, a result in agreement with that of Soliman et al. (2009) and El-Shafey et al. (2011). It has been reported that the increase in plasma ALT and AST activities in abamectin treated rats may be due to the decreased catabolism rate of these enzymes in plasma (Kramer,1989).

The above findings were confirmed by histopathological changes in the liver under the intoxication effect of abamectin. Abamectin caused a marked damage of the liver tissue in the form of edema, dilation of Disse's space, necrobiosis of hepatic cells and chronic venous congestion.

The increase in AST may impair the liver to be more susceptible to other pathogen/toxicants (Chamulitrat and Spitzer, 1996: Nayak et al., 1996). AST is an indicator of liver damage in clinical studies. During hepatocellular damage, AST was found to be secreted into the blood (Kalander, 2009). In damage cells, it was found that these enzymes leak into the blood stream (Mansour and Mossa, 2010).

The elevation in the liver enzyme activities may be due to liver dysfunction and altered membrane permeability enzyme leakage in the blood with a consequent reduction in enzyme biosynthesis (Mansour and Mossa, 2010). In the present study the elevation in the ALT and AST plasma level may be due to hepatotoxicity causing permeability alteration and leakage of lysosomal enzymes enhancing the release of enzymes (Choudhary et al., 2003), due to liver damage by abamectin. This seems to be the same in the present study, since abamectin damaged the hepatocytes of rats as illustrated above.

It was found that abamectin combined with vitamin C caused a significant increase in plasma ALT and AST, but, abamectin combined with vitamin E led to a nonsignificant increase in the plasma level of ALT and a nonsignificant decrease in the plasma level of AST. Also, abamectin combined with both vitamins C and E caused a nonsignificant decrease in the plasma level of ALT and a nonsignificant increase in the plasma level of AST. These results were confirmed by findings that vitamin C and E ameliorated the effects of abamectin on the liver tissue which most of them appeared normal except few of them still showed dilation of blood

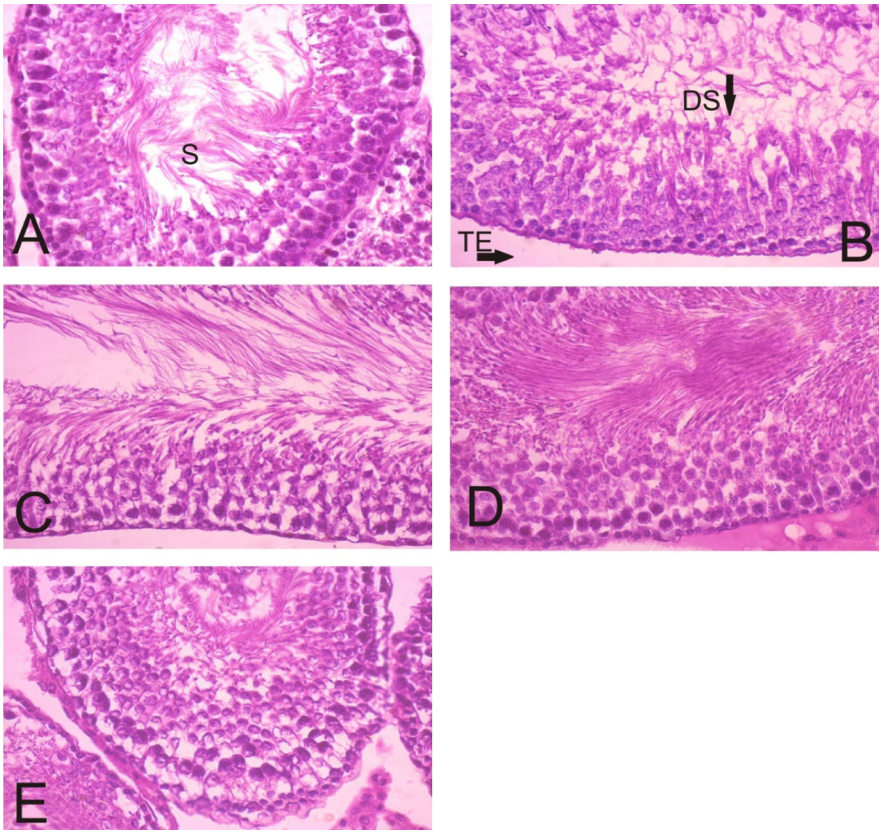

Plate 3 Photomicrograph of testis section from rats of the control group (G1) showing normal morphological structure with all successive stages of spermatogenesis, and lumen filled with spermatozoa (S), (A); of the second group (G2) which was treated with abamectin showing intertubular edema (TE) and degeneration in some spermatogenic cells (DS) with the presence of few number of spermatozoa, (B); of the third group (G3) which was treated with abamectin combined with vitamin C showing normal histological structure, (C); of the fourth group (G4) which was treated with abamectin combined with vitamin E showing normal histological structure. (D); of the fifth group (G5) which was treated with abamectin combined with vitamin C plus vitamin E showing normal histological structure, (E). (H&E) (40×).

vessels and inflammation. Here, we can conclude that vitamin C and vitamin E act as antioxidants to some extent. However, the findings that vitamin C combined with vitamin E did not remove the toxic effect of abamectin on the histological structure of liver, and did not cause a decrease in the plasma level of ALT and AST led us to conclude that abamectin still has a toxic effect even in the presence of vitamins C and E which are known as antioxidants (Uzun et al.,2009).

The results obtained in the present study also revealed that abamectin caused a significant increase in the plasma level of acid phosphatase (AP). These results are in coincidence with those of Eissa and Zidan (2010), who reported that abamectin caused an increase in the activity of AP in the male albino rats. It has been stated that the elevated AP activity may be associated with the cell disintegration resulting from pesticide treatment, thus suggesting prenecrotic changes in the liver (Saigal et al.,1982). The above results were confirmed by histopathological changes induced by abamectin including edema, dilatation of Disse's space and necrobiosis of some hepatic cells. The findings that abamectin combined with vitamin E resulted in a highly significant decrease in plasma level of AP are in accordance with those obtained by Uzun et al.,(2009) who reported that organophosphorous compounds exert their deleterious effects by promoting destructive oxidation of lipids, proteins and DNA within the hepatic cells.

The present study demonstrated that abamectin caused a highly significant increase in the plasma level of glucose. These results are in disagreement with those obtained by Eissa and Zidan (2010), who reported that abamectin administration in male albino rats resulted in the decrease in glucose concentration. The results obtained for abamectin indicated that abamectin resulted in a marked decrease in the carbohydrate in the liver tissue. It has been reported that the rise in the blood glucose may indicate disrupted carbohydrate metabolism due to enhanced breakdown of liver glycogen, possibly mediated by an increase in adrenocorticotrophic and glycogen hormones and/or reduced insulin activity (Raja et al., 1992). It was suggested that the hyperglycemia and the decrease in the liver glycogen observed in abamectin treated group may be due to the inhibition of pancreatic B-cell activity and lack of sufficient insulin secretion (Yousef,2004). The findings that abamectin combined with vitamin C, and abamectin combined with vitamin C plus vitamin E caused a highly significant decrease in the plasma level of glucose and exhibited normal distribution of carbohydrates in the hepatic cells indicates that vitamin C and vitamin E act as antioxidants to protect the liver against

toxic effects of abamectin. However, we can conclude that abamectin still has its toxic effect on the hepatic tissues, since vitamins C and E did not remove the histopathological changes exerted by abamectin on the liver.

In this study, it was also found that abamectin caused a significant increase in the plasma level of total protein and albumin. These results are in contrast to that obtained by Eissa and Zidan (2010) and Abd-Elhady and Abou-Elghar (2013) who reported that abamectin caused a decrease in the level of total protein and albumin in male albino rats. The changes in the level of protein and glycogen suggest disturbance of protein synthesis as a result of impaired hepatic function (Celia and Wilkinson,1973). Hyperalbuminemia (increased albumin) is a liver disorder thought to be a consequence of increased hepatic synthesis of albumin as a result of toxic effects of abamectin. The findings that vitamin C combined with vitamin E resulted in a decrease in the plasma level of total protein and albumin suggest that vitamins C and E as antioxidants ameliorate the toxic effect of abamectin on the hepatic tissues. However, it should be noted that vitamin C combined with vitamin E ameliorates the toxic effects of abamectin on the liver enzymes (ALT, AST, AP) glucose, total proteins and albumin, but they did not remove the histopathological changes caused by abamectin in the liver tissue. So, it can be concluded that abamectin may have acted directly on the liver tissue and affected the hepatic enzyme biosynthesis and may be the antioxidant action of vitamins C and E is not enough to remove the toxic effect of abamectin on the hepatic tissues. So, the actions of abamectin, vitamin C and vitamin E need more work on the liver structure and function.

Kidney

The present study showed obvious significant increase in the plasma level of uric acid, urea and triglycerides with abamectin administration, whereas, abamectin caused a significant decrease in the plasma level of creatinine and cholesterol. The increase in the uric acid activity is in agreement with that obtained by Abd-Elhady and Abou-Elghar (2013), Eissa and Zidan (2010). But, the decrease in the plasma level of creatinine is opposite to that recorded in albino rats where abamectin caused an increase in the blood creatinine concentration (Abd-Elhady and Abou-Elghar, 2013). The elevation of uric acid, urea and triglycerides concentrations may be attributed to the reduction in the glomerular filtration in the kidney. Such an increase also reflects the dysfunction of the kidney tubules (Walmsley and White,1994). Also, the increase in uric acid concentration is a demonstration of impaired kidney function since the organ primarily exerts urea in the urine. These results were confirmed by the histopathological changes caused by abamectin including necrobiotic changes in the renal glomerular and tubules in the renal cortex associates with hemorrhage. The decreased level of creatinine and cholesterol as a result of abamectin administration may be attributed to the dysfunction of kidney as creatinine is the end product of protein catabolism. This is supported by results obtained in the present study, in which abamectin was noted to increase the level of total protein due to its inhibited catabolism.

The results obtained showed that no histological changes were noted in the kidney under the effects of abamectin combined with vitamin C, since no significant changes in the plasma level of urea, uric acid, creatinine and cholesterol were observed, but they caused a significant increase in the level of triglycerides. However, abamectin treated group with vitamins C, E and abamectin combined with vitamin C and vitamin E ameliorated the previous histological and biochemical parameters.

It has been reported that urea in the blood is a consequence of its production rate during amino acid catabolism and its excretion by rate kidney. Creatinine concentration in blood is a result of balance between creatinine production by the muscle and excretion by kidney. So, it can be concluded that abamectin increased the catabolism of the biochemicals to meet the enhanced energy demand of animals under stress or their reduced synthesis due to impaired tissue function, whereas vitamins C and E by their antioxidant action may protect the kidney tissue against the oxidative stress of abamectin on the kidney tissues.

It should be noted that the information about the effect of abamectin on the plasma level of urea, cholesterol and triglycerides, and the ameliorative effect of vitamins C and E in the literature are very scarce.

Testis

It has been reported that abamectin toxicity led to damage in male reproductive system in rats (Elbetieha and Da'as, 2003). This damage include congestion of blood vessels, hemorrhage at areas surrounding seminiferous tubules, increased amount of connective tissue between seminiferous tubules and immature spermatids in the lumen of seminiferous tubules. All of these changes resulted in reduction in male fertility (Elbetieha and Da'as, 2003). Abamectin exposed rats showed a significant testicular damage including degenerative seminiferous tubules with disrupted cellular organization and a decrease in sperm count (Celik-Ozenci et al., 2011). The oral administration of abamectin in male albino rats resulted in degeneration of spermatogonia lining seminiferous tubules, marked degenerative and necrosis of spermatogonia cells lining seminiferous tubules associated with peritubular edema and lumen contains a decreased number of spermatogenic elements (Abd-Elhady and Abou-Elghar,2013). The above results are in agreement with the results in the present study, since oral administration of abamectin induced intertubular edema, degeneration in some spermatogenic cells and a decrease in the number of spermatozoa.

Therefore, it can be concluded that abamectin acts directly on the testes and affects the androgen biosynthesis pathway, since oral administration of abamectin caused degeneration in spermatogenic cells associated with few number of spermatozoa. It has been reported that abamectin impair male reproduction function by reducing sperm counting and motility (Chitra et al.,2003; Nagoula et al.,2007; Mathur and D'cruz, 2011). Also, it has been stated that abamectin *in vivo* caused a decrease in sperm motility that could induce the spermatogenic failure (Celik-Ozencic et al., 2012).

The administration of abamectin combined with vitamin C, abamectin combined with vitamin E and abamectin combined with vitamins C and E had a recovery effect on the histological picture of testes. It means that vitamins C and E as antioxidants remove completely the toxic effects of abamectin on the histological structure of rat testes.

In conclusion, the results of this study demonstrate that biocide, abamectin had toxic effects on biochemical function which correlates well with the histopathological changes of liver and kidney. Also, it had a toxic effect on the histological structure of rat testis. But, antioxidant vitamins C and E are able to improve hepatic and renal function by ameliorative oxidative stress induced by abamectin. Moreover vitamins C and E had ameliorated the toxic effect on the histological changes induced by abamectin in liver, kidney and testis, but not completely in the liver tissues. So, the data in this study revealed a risk of target organs damage during the exposure to insecticide (abamectin).

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